Aggregates of two-dimensional vesicles: Rouleaux and sheets

P. Ziherl

Department of Physics, University of Ljubljana,

Jadranska 19, SI-1000 Ljubljana, Slovenia

Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

(Dated: February 1, 2008)

Abstract

Using both numerical and variational minimization of the bending and adhesion energy of two-dimensional lipid vesicles, we study their aggregation, and we find that the stable aggregates include an infinite number of vesicles and that they arrange either in a columnar or in a sheet-like structure. We calculate the stability diagram and we discuss the modes of transformation between the two types of aggregates, showing that they include disintegration as well as intercalation.

PACS numbers: 87.16.Dg, 87.18.Ed, 87.18.La

Since Hooke's *Micrographia*, shapes of aggregates of simple cells have fascinated biologists, physicists, and mathematicians alike. In the central part of Thompson's classic *On Growth And Form* devoted to the "forms of cells" and "cell-aggregates" [1], the main concept used to interpret the striking regularity of many aggregates is surface tension which forces cells to minimal-area configurations. Of course, Thompson viewed surface tension merely as a mesoscopic manifestation of the complex biochemical apparatus and the internal structure of a cell. Similar ideas are still being explored: Recently, cells of the developing Drosophila retina were shown to pack just like soap bubbles [2]. Another concept related to some biological cells (e.g., the red blood cell [3]) is that of a vesicle formed by a closed lipid bilayer membrane [4]. The structure of a vesicle aggregate held together by intermembrane attraction is determined by both adhesion and membrane elastic energy rather than by surface energy alone as in a soap froth.

Regardless of their mechanical framework — soap bubble [2], lipid vesicle [4], or a Potts Hamiltonian [5] — phenomenological models of cell aggregates are most transparent within the context of undifferentiated systems with distinct geometry, e.g., early embryonic stages or layered tissues of epithelial sheets. Some epithelial tissues consist of a single layer of prismatic cells and their salient geometrical feature is their cross-section, essentially a polygonal partition of a plane. In many cases, the structural statistics of two-dimensional partitions obey simple empirical relationships such as the Lewis law first observed in cucumber epidermis [6] and the Aboav-Weaire law [7]. In a soap-froth-like partition, the link between its structure and the local equilibrium is embodied in the Plateau rules, one of the premises used to clarify the above laws [8, 9]. On the other hand, no such rules exist for partitions formed by vesicle aggregates: Their energy functional is more complicated than that of a soap froth, and their minimal-energy configuration is unknown.

In this paper, we study aggregates of two-dimensional vesicles as a model which could elucidate some aspects of the in-plane structure of certain simple layered tissues. By employing the theory of elasticity combined with the contact-potential intermembrane attraction, we build on existing insight into the shape of free two-dimensional vesicles [10] and their adhesion on flat substrates [11]. We focus on vesicles of identical area and perimeter, and we find that the stable aggregates consist of infinitely many vesicles. We analyze both columnar and sheet-like infinite aggregates, discuss the modes of transformation between them, and construct the stability diagram.

The model used here is based on the two-dimensional version of the lipid bilayer bending energy $W_b = (K/2) \oint C^2(s)\,ds$, where K is the two-dimensional bending constant and C is the local curvature [12]. The integral is evaluated along the contour of a vesicle subject to two constraints, that of a fixed perimeter L and that of a fixed enclosed area A. The characteristic length scale is given by R_c, the radius of a circle of perimeter L, such that the reduced vesicle perimeter is normalized to $\oint ds/2\pi R_c = 1$ and the reduced area $a = \int dA/\pi R_c^2$ ranges between 0 and 1. The aggregate energy includes the bending energies of all members and the adhesion energy proportional to the total length of the contact lines: $W_a = -\Gamma \sum_i \int_{\text{contact}} ds_i$ [11]. Here Γ is the adhesion strength; the sum runs over all contact lines and the integral over their contours. The energy scale is given by $\pi K/R_c$, the bending energy of the reference vesicle with $a = 1$, and the reduced adhesion strength is $\gamma = \Gamma R_c^2/2K$.

By minimizing the energy numerically using Surface Evolver [13], we first reproduce the shapes of free vesicles [10]. For $a = 1$, the vesicle is a circle, for $0.8 \lesssim a < 1$ it adopts an ellipsoidal shape, for $0.27 < a \lesssim 0.8$ it is characterized by two invaginations on its long sides, and for $a < 0.27$ it consists of two tether-connected buds (Fig. 1). Their elongated shape determines the way vesicles preferentially stick to each other — the contact line of a pair is longest if the vesicles' long axes are parallel.

This is confirmed by the doublet shapes; a typical example is shown in Fig. 1. The doublets show that a columnar arrangement (briefly called a *rouleau* due to its equivalence to the characteristic red blood cell aggregate) is a good candidate topology. We analyzed its stability as both number of members and adhesion strength are varied, finding that the threshold for rouleau formation decreases with the number of members. For example, the columnar doublets, triplets, and quadruplets of vesicles with $a = 0.5$ are stable for reduced adhesion strengths beyond $0.061, 0.052$, and 0.048, respectively; the extrapolated aggregation threshold for the infinite rouleau is at $\gamma = 0.042$. Thus the stable rouleau consists of infinitely many vesicles, which is plausible: The more members in an aggregate, the longer the average total contact line per vesicle and the lower the threshold.

The rouleau is not the only possible arrangement of vesicles but our numerical studies of other topologies of triplets and quadruplets at various a and γ strongly indicate that these are relevant exclusively in finite aggregates above a certain adhesion strength which increases dramatically as the vesicle area is decreased. As an illustration, we note that even in vesicles of area as large as 0.95, the trefoil is stable compared to the rouleau triplet only
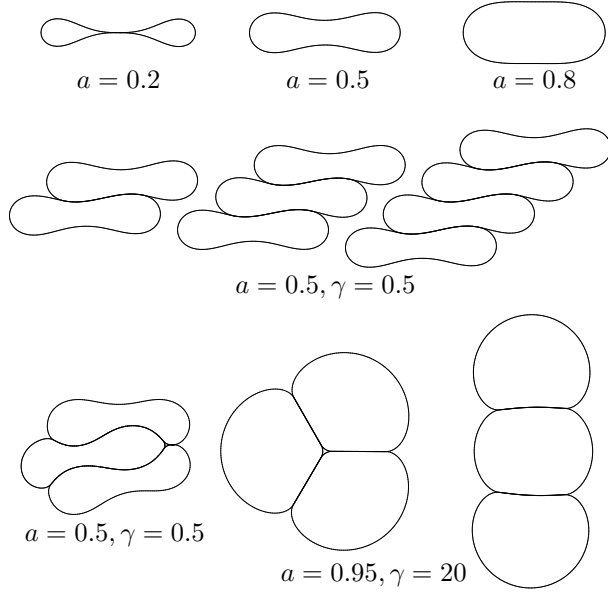
FIG. 1: Representative numerically obtained vesicle and aggregate shapes. Top: Free vesicles of small ($a = 0.2$), intermediate ($a = 0.5$), and large reduced areas ($a = 0.8$). Center: Columnar aggregates of 2, 3, and 4 vesicles for $a = 0.5$ and reduced adhesion strength $\gamma = 0.5$. Bottom: The energy of a trefoil exceeds that of a columnar triplet unless a and γ are both large. The $a = 0.5, \gamma = 0.5$ trefoil is metastable, its $a = 0.95, \gamma = 20$ counterpart is stable. Also shown is the metastable columnar triplet for $a = 0.95$ and $\gamma = 20$.

for γ beyond 10.9 which is well above the aggregation threshold ≈ 0.5 as argued below. But the total contact line of the middle vesicle in the $a = 0.95$ rouleau triplet is longer than the average total contact line per vesicle in the trefoil regardless of γ, and thus the energy of a rouleau with a large enough number of members is always lower than that of a set of trefoils. This supports the conclusion that the stable rouleau is infinite.

Fig. 1 shows that the contact lines of vesicles in an infinite rouleau can be either sigmoidal or flat [14]. This is exemplified by the central pair of vesicles in the numerically obtained ten-member $a = 0.2$ rouleaux (Fig. 2). The outer contact lines of the pair are almost parallel both at $\gamma = 25$ where they are sigmoidal and at $\gamma = 40$ where they are almost flat, which means that the vesicle pair may serve as the repeat unit of an infinite stack.

Presently, a complete numerical analysis of the structure of vesicle aggregates is well beyond reach even with advanced tools such as Surface Evolver. Instead, we use the above
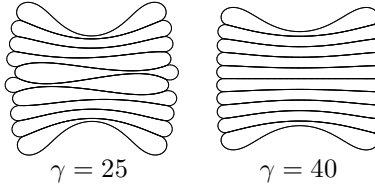
FIG. 2: Rouleaux of ten $a = 0.2$ vesicles for $\gamma = 25$ and 40. In both cases, the outer contact lines of the central pair are nearly parallel, showing that the pairs can serve as sigmoid-contact and flat-contact repeat units of an infinite rouleau.

cues to construct a variational model of an infinite rouleau. We describe the sigmoid-contact vesicle by two circular caps that together make up a full circle and two identical one-wave sinusoids combined into a smooth contour (Fig. 3), which can be done either such that the sinusoids are parallel (which gives an S-shaped vesicle) or such that their phases are opposite (which gives a pear-shaped vesicle). The model flat-contact vesicle consists of two identical straight lines connected by half-ellipses. — Using the fixed-area and fixed-perimeter constraints, two out of the three parameters of the model shapes can be expressed in terms of the remaining one by resorting to analytical approximations of the elliptic integral, and the equilibrium value of the free parameter is then adjusted so as to minimize the total energy.
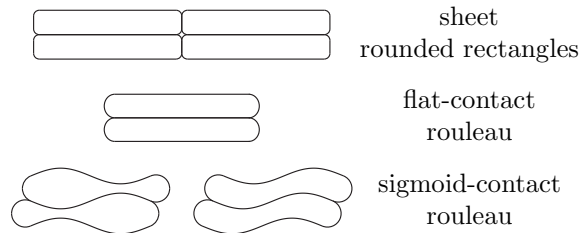

FIG. 3: Equilibrium $a = 0.4$ building blocks of the model sheet-like aggregate (introduced below; $\gamma = 45$), flat-contact rouleau ($\gamma = 19$), and pear-shaped and S-shaped sigmoid-contact rouleau ($\gamma = 1$). — The regular tiling shown is a special example of the sheet geometry; sheets are generally structurally disordered as the energy of the rounded convex polygonal model vesicle does not depend on its precise shape.

This model reproduces the continuous transition from the sigmoid-contact to the flat-contact repeat unit implied by the numerically calculated shapes of the ten-member rouleau

for adhesion strengths between 25 and 40. As γ is increased beyond the transition, the model flat-contact rouleau undergoes a telling transformation: The length of the contact line and the eccentricity of the cap of a vesicle both grow such that its shape approaches a rounded rectangle (Fig. 3). This suggests that at large enough γ, a sheet-like vesicle arrangement with two-dimensional connectivity may be preferred to a rouleau. To explore this possibility, we construct a model sheet-like aggregate using convex vesicles with a contour consisting of straight contact lines and circular arcs of identical radii (Fig. 3). We find that the reduced energy of this aggregate is $\sqrt{2\gamma}\left(2 - \sqrt{2\gamma}\right)$ independent of a, number of sides, and relative sizes of the straight sections and the internal angles; the equilibrium curvature of the rounded corners is $\sqrt{2\gamma}$ in agreement with the boundary condition at the edge of the contact zone [15].

Using all model structures, we can delineate the complete stability diagram of the aggregates; by comparing them to the numerically calculated free vesicle shapes, we also outline the aggregation threshold. As anticipated, the sequence observed upon increasing adhesion strength is free vesicle/rouleau/sheet unless the reduced vesicle area is too small or too large (Fig. 4); for $a < 0.45$, the rouleau region is subdivided into the sigmoid-contact and the flat-contact part. For $a < 0.27$ where the free vesicles consist of two tether-connected buds, the small-γ rouleaux are more complicated than those studied here; we did not explore this regime in detail.

Given that the rounded polygonal model vesicles are energy degenerate, the sheet-like aggregate should consist of coexisting triangles, tetragons, pentagons, hexagons, heptagons, etc. conceivably forming orientationally ordered structures. Such coexistence is characteristic of the most part of the sheet region. However, as a increases the set of possible shapes is gradually narrowed and the sheet becomes more ordered, which is illustrated by the maximal vesicle areas where a periodic tiling with three-, four-, and sixfold coordination is possible at a given γ (Fig. 4). The largest reduced vesicle area which supports a sheet corresponds to a rounded regular hexagon: Beyond this point, vesicles can only form flat-contact rouleaux.

A potentially relevant feature of a sheet-like aggregate is the relative intervesicular area I defined as the fraction of the plane not covered by the vesicles. I can be evaluated easily for regular tilings: To lowest order, $I = \beta/\gamma a$ where β equals $(2\sqrt{3} - \pi)/2\pi \approx 0.051$ for a tiling of equiangular convex hexagons and $(4 - \pi)/2\pi \approx 0.137$ for a rectangular tiling (suggesting that if there were a reason that I should be minimized, partitions with regular 3-way vertices would be preferred). I is typically small — for equiangular hexagons, its
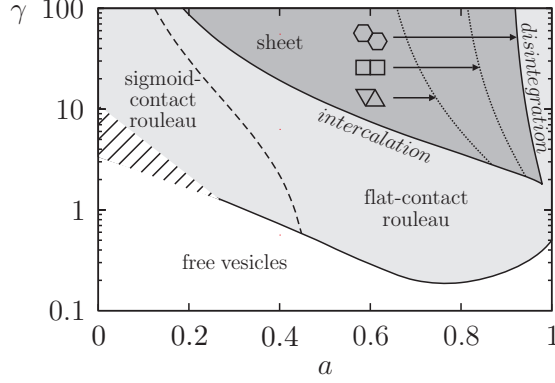
6

FIG. 4: Stability map of adhering two-dimensional vesicles is divided into regions of free vesicles, infinite rouleaux, and infinite sheet-like aggregates. The sigmoid-contact/flat-contact rouleau transition is shown by the dashed line; the large-area limit of stability of rouleaux is infinitesimally smaller than 1 as at $a = 1$ the vesicles can only be free. The largest reduced areas where periodic sheet tilings with three-, four-, and sixfold coordination are possible correspond to the two dotted lines and the right-hand boundary of the sheet region, respectively, labeled by the repeat units free of rounded corners for clarity. Also indicated are the two modes of the sheet/rouleau transition described below. — For $a < 0.27$, a free vesicle reduces to two tether-connected buds, and in the parameter space qualitatively outlined by the crossed region such vesicles form aggregates more complex than those discussed here.

upper limit reached at $a = 0.98$ and $\gamma = 1.8$ is $I = 2.9\%$.

On a quantitative note, the aggregation threshold is overestimated in our analysis. For example, the numerically obtained value for vesicles with $a = 0.5$ is at $\gamma = 0.042$ rather than at $\gamma = 0.46$ as shown in Fig. 4. This discrepancy is expected as the variational aggregate energies are too large; we estimate that for $\gamma \gtrsim 0.5$ they depart from the exact values by less than 10%. However, we stress that the error is a rapidly decreasing function of γ. This is illustrated by the finite rouleaux with $\gamma = 25$ and 40 shown in Fig. 2 where the central vesicles are clearly described rather well by the ansatz shapes compared to vesicles in the $\gamma = 0.5$ linear aggregates (Fig. 1); the numerically calculated transition between the two types of the 10-member rouleau with $a = 0.2$ is at $\gamma \approx 32$ very close to $\gamma = 31$ as predicted variationally for the infinite rouleau. Thus the model performs well at large γ and we trust that the sheet/rouleau boundary shown in Fig. 4 is quite accurate.

The sheet/rouleau transition is a very interesting aspect of the stability diagram, and it

can be accomplished either by disintegration of a sheet into several rouleaux or by vesicle intercalation which transforms a sheet into a single rouleau. The first mode is preferred along the right-hand boundary of the sheet region where the only possible vesicle type is the rounded hexagon and the sheet can break up into parallel rouleaux along a lattice axis. On the other hand, at the left-hand/bottom boundary the sheet is disordered so the vesicles cannot dissolve easily into separate rouleaux. But they can intercalate, thereby gradually decreasing the extent of a sheet along the average long axis of vesicles. Intercalation is accompanied by vesicle alignment and effectively continuous: Relocation of vesicles is facilitated by the degeneracy of their energy so that they may change their shape at fixed area and perimeter as appropriate at no cost.

These results may be related to an important mechanism of cell rearrangement known as convergent extension [16] whereby cells in a layered structure i) undergo in-plane elongation, ii) align, and iii) intercalate such that the tissue is extended in the direction perpendicular to the long axes of cells. The physical side of this process can be described in terms of the differential adhesion hypothesis by assuming that the en-face cross-section of cells is elongated, say rectangular with 2 long and 2 short sides, and that the adhesion strengths for long-long, long-short, and short-short contacts are all different [17]. Under certain conditions, this gives a minimal-energy aggregate that is orientationally ordered and elongated normal to cell axes, thus reproducing the final stage of convergent extension. But the simplest microscopic basis of differential adhesion would be a variation of the density of anchoring junctions across the cell sides, and there is no solid evidence either for or against this [17].

The stability diagram (Fig. 4) offers an alternative explanation: Mechanically, convergent extension could be the process of sheet/rouleau transition induced by a decrease of either adhesion strength or reduced area of the en-face cell cross-section, whereby the sheet would seek the energy minimum by transforming into a rouleau via intercalation. In real tissues, convergent extension is certainly not driven solely by the interplay of the intermembrane adhesion and cell elastic energy as a passive, non-specific morphogenetic force; the sheet/rouleau transition may be either promoted or impeded by the action of cytoskeletal machinery and cell internal structures as well as by the interaction of the cell with the extracellular matrix and the surrounding tissues. Nonetheless, our scenario is consistent with several subdominant features of the process unaccounted for by the differential adhesion model. For example, convergent extension can occur in absence of changes in the cell

8

shape [18] (in this case, it could be induced by a decrease of adhesion strength), and it can be accompanied by the onset of disorder where six-fold cell coordination is replaced by patterns including a range of polygonal shapes [19] (which happens in the sheet-like aggregate as the vesicle reduced area is decreased). This suggests that the differential adhesion model [17] can be regarded as an effective, coarse-grained version of our theory where the vesicle elastic energy is absorbed in an anisotropic adhesion constant.

In conclusion, our analysis of adhering two-dimensional vesicles shows that the structure of minimal-energy aggregate is simplified by the fact that the stable aggregates, both linear and sheet-like, are infinite. The main results should be qualitatively insensitive to a moderate vesicle polydispersity, and they call for a generalization to three-dimensional vesicles.

[1] D. W. Thompson, *On Growth And Form* (Dover, New York, 1992).

[2] T. Hayashi and R. W. Carthew, Nature **431**, 647 (2004).

[3] S. Svetina and B. Žekš, Anat. Rec. **268**, 215 (2002).

[4] U. Seifert, Adv. Phys. **46**, 13 (1997).

[5] J. A. Glazier and F. Graner, Phys. Rev. E **47**, 2128 (1993).

[6] F. T. Lewis, Anat. Rec. **38**, 341 (1928).

[7] D. Weaire and N. Rivier, Contemp. Phys. **25**, 59 (1984).

[8] N. Rivier and A. Lissowski, J. Phys. A **15**, L143 (1982).

[9] M. A. Peshkin, K. J. Strandburg, and N. Rivier, Phys. Rev. Lett. **67**, 1803 (1991).

[10] S. Leibler, R. R. P. Singh, and M. E. Fisher, Phys. Rev. Lett. **59**, 1989 (1987).

[11] U. Seifert, Phys. Rev. A **43**, 6803 (1991).

[12] P. B. Canham, J. Theor. Biol. **26**, 61 (1970).

[13] K. Brakke, Exp. Math. **1**, 141 (1992); Surface Evolver is available at http://www.susqu.edu/facstaff/b/ brakke/evolver/evolver.html.

[14] We tacitly assume that any two vesicles touch at a single contact line, which is true at large enough reduced areas. For $a < 0.27$ where a free vesicle consists of two tether-connected buds,

this need not be the case: At small adhesion strengths, vesicles in an aggregate are not too different from the free ones, and each bud should form a separate contact with another vesicle. But at large γ, the vesicles are flattened out and the aggregates are qualitatively the same as for $a > 0.27$, which is shown in Fig. 2.

[15] J. Derganc, B. Božič, S. Svetina, and B. Žekš, Biophys. J. **84**, 1486 (2003).

[16] R. Keller, Science **298**, 1950 (2002).

[17] M. Zajac, G. L. Jones, and J. A. Glazier, Phys. Rev. Lett. **85**, 2022 (2000).

[18] K. D. Irvine and E. Wieschaus, Development **120**, 827 (1994).

[19] J. A. Zallen and R. Zallen, J. Phys. Condens. Matter **16**, S5073 (2004).